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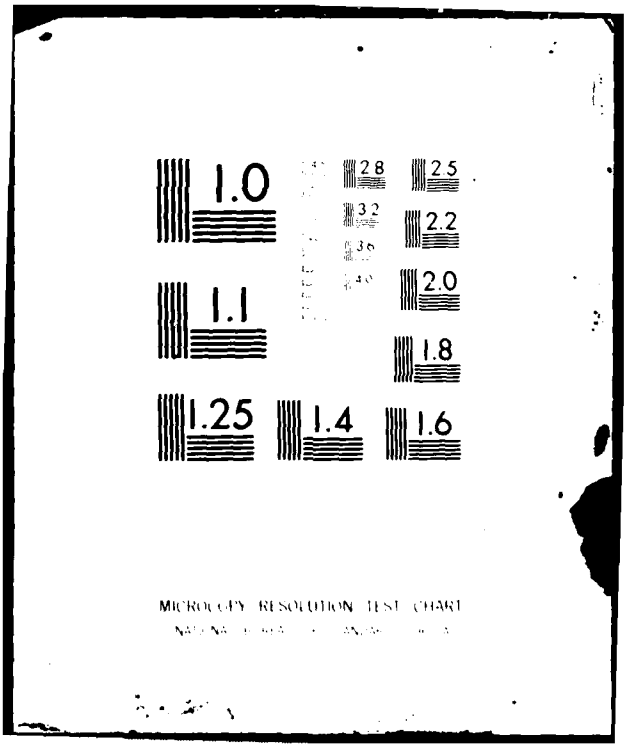
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Report SAM-TR-81-39

EFFECTS OF TEMPERATURE AND ORIENTATION OF AN INTACT RABBIT LENS ON THE POLARIZED RAMAN SPECTRA

Dwaine M. Thomas, Ph.D.

December 1981

Final Report for Period August 1979 — January 1980

USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
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NOTICES

This final report was submitted by personnel of the Laser Effects Branch, Radiation Sciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas, under job order 7757-02-53.

When U.S. Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Effects of temperature and orientation of an intact rabbit lens on the polarized Raman spectra are discussed. Regardless of rabbit lens orientation with respect to incident/scattered exciting laser beams, the resultant polarized Raman spectra are essentially identical. The results indicate that the lens is optically isotropic. Temperature at 100°C seems to have no effect on protein conformation within the lens.

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EFFECTS OF TEMPERATURE AND ORIENTATION OF AN INTACT RABBIT LENS ON THE POLARIZED RAMAN SPECTRA

INTRODUCTION

Recently a number of papers have dealt with the chemical makeup of the crystalline lens in various animal species, including humans (1-3). These results have essentially been concerned with the spectroscopic properties of lenticular proteins; namely, the α, β, and γ crystallins. Raman spectroscopy has been the primary technique used in elucidating conformational properties of these proteins and in determining their vibrational characteristics and corresponding amino acids (4,5). Little information exists, however, regarding polarization properties with respect to orientation of the intact lens. In a previous paper we investigated the UV-laser radiation effects on a rabbit lens (6); in the course of that work, baseline spectral properties were studied. The purpose of this paper is to present orientational effects of a rabbit lens on the polarized Raman spectra and the effect of high temperature on the conformation of proteins of the rabbit lens. Our aim is to gain a better understanding of the lens transmission properties as well as of temperature and radiation effects on the normal functioning lens.

MATERIALS AND METHODS

Subjects for these experiments were mature Dutch-belted rabbits (2-4 kg) with healthy corneas and lenses. Just before the animal was to be sacrificed, a Nikon slit lamp was used to examine the eyes to insure that normal lenses were present. The rabbits were sacrificed with a lethal dose of Barb-Euthol (0.22 cc/kg, Haver-Lockhart); the eyes were enucleated; and intact lenses were excised. Lenses were examined under a surgical microscope to assure that no damage had occurred and were then transferred to the optical cell. The quartz cell used to hold the lenses has been discussed previously (6). Resting in the axial position, the lens could be easily changed to equatorial and slanted (45°) orientations. For the high-temperature measurements, a lens was deposited in a test tube and immersed in boiling water until 100°C was maintained inside the test tube for

5 minutes. The lens immediately turned opaque and white, yet remained intact. The lens was removed and sliced into several sections; a cortical section (a region outside the central nucleus) was used for the Raman measurements. This particular section was solid enough to be mounted in a solid sample holder; a 90° scattering geometry was used.

Raman spectra were recorded using a Cary model 82 spectrophotometer with a Spectra Physics 170-03 argon-ion laser as an excitation source; the 514.5-nm laser line was used for all measurements. The frequency accuracy for all spectra, based on calibration results, is $\pm$ 2 cm^{-1}. For the polarization measurements, a polarizer and polarization scrambler were inserted in the optical train to obtain spectra parallel and perpendicular to the incident electric vector, labelled ($A_{\parallel}$) and ($B_{\perp}$) respectively. Laser power at the sample was measured using a Cary power meter; power was varied between 235 and 300 mW. No evidence suggested that the intact lens was affected by the probing laser beam or its container environment; thus, we assumed that the intact lens did not undergo any unintended changes.

RESULTS AND DISCUSSION

Lens Orientation

The three orientation spectra for the intact rabbit lens are shown in Figures 1-3. Several rabbit lenses displayed similar spectral patterns and intensity distributions. The region between 200 and 1800 cm^{-1} was recorded at a higher sensitivity level (gain) because of the overall intensity weakness compared to the region from 2000 to 4000 cm^{-1}. Table 1 lists the observed frequencies for the axial position of the rabbit lens.

A detailed discussion of the assignments of the observed bands for the intact rabbit lens has been published elsewhere (6). Essentially, the spectral range from 200 to 4000 cm^{-1} can be discussed as two separate regions; the high frequency region, 2000-4000 cm^{-1}, and the low frequency region, 200-1800 cm^{-1}.

Three important types of vibrations are in the 2000-4000-cm^{-1} region. These are the S-H stretch at 2575 cm^{-1}, the C-H stretch at 2935 cm^{-1}, and the

water structure at 3280 and 3400 cm^{-1}. In comparing the spectra for the three orientations (axial, equatorial, and slanted), we find no difference among them in terms of polarization, band appearance, or intensity distribution. The C-H stretching mode at 2935 cm^{-1} appears as the strongest band in the entire spectrum. In the perpendicular position ($B_\perp$), another component appears at 2965 cm^{-1}. At 3060 cm^{-1} another C-H stretching mode appears; this, too, has a component at 3040 cm^{-1} which appears in the ($B_\perp$) spectrum. These C-H vibrations do not change among the three orientations. The weak S-H vibration at 2575 cm^{-1} also does not change noticeably. The final remaining important bands are due to water vibration. The modes at 3400 and 3280 cm^{-1} are both polarized with a third component appearing at 3450 cm^{-1} in the $B_\perp$ spectrum; these bands also change very little for different orientations. The water-band system, however, shows a slight intensity difference between the equatorial and slanted positions. The 3400- to 3280-cm^{-1} bands are strongest in the slanted position and weakest in the equatorial position. This very small difference could be due to differences in gain or overall scattering efficiency in the two orientations. The scattering volume is believed to be almost the same for all three orientations in the cortical section, but slight local differences could be in the water concentration. In all, the polarization results are essentially identical for the three orientations, with the possibility that local water-concentration differences might produce very slight changes.

In the region between 200 and 1800 cm^{-1}, the amino acid and protein backbone vibrations occur. Below 400 cm^{-1}, no vibrational modes are observed (unpublished results). This implies no lattice-mode vibrations, and consequently no lattice-mode structure in the crystalline lens. In this region, as in the high frequency region, all three orientation spectra are essentially the same in terms of polarization and intensity distribution. A few exceptions show slight differences in intensity distribution, and these will be discussed.

Two weak bands that appear at 620 and 644 cm^{-1} are assigned to the amino acids, phenylalanine and tyrosine respectively; these two bands are unique in that they are depolarized. The intensity ratio $A_\parallel / A_\parallel$ between 644 and 620 cm^{-1} varies in the three spectra: axial = 1.67; slanted = 1.33; and equatorial = 1.00. If the concentration distribution of phenylalanine and tyrosine is fairly constant throughout the lens, this ratio difference could indicate local orientational effects of the two ring systems. Both of these vibrations are out-of-plane ring

deformations, and their intensity should depend on in-plane/out-of-plane orientation effects. It is feasible that phenylalanine changes from an in-plane axial orientation, where the plane of the ring is parallel to the incident electric vector, to an out-of-plane equatorial orientation, where the plane of the ring is perpendicular to the incident electric vector, and as a result, the vibration gains intensity. This effect, however, is not due to orientation of the lens as a whole but to a local effect. At 759 cm^{-1} a tryptophan band appears that remains constant in intensity and polarization for all three positions.

A series of bands forming a triplet appears at 870, 855, and 830 cm^{-1}. These are due to tryptophan (870) and tyrosine (855, 830) respectively. The overall band intensity for the triplet is slightly stronger in the axial than the equatorial position. The same slight intensity changes occur for the Amide III band at 1235 cm^{-1}; this band decreases from the axial to equatorial position. These changes are considered too small to be of significance.

For the phenylalanine bands at 1207 and 1603 cm^{-1}, there is a marked increase in intensity for the equatorial position as compared to the axial. The same is true for the 1003-cm^{-1} phenylalanine band. A comparison between the 1003- and 1669-cm^{-1} (Amide I) bands shows that the difference between the intensities of the two increases from the axial to the equatorial position: axial = 19, slanted = 20, and equatorial = 29. A trend is indicated by the 1003-, 1207-, and 1603-cm^{-1} phenylalanine bands: the vibrations increase in intensity when oriented in the equatorial position. Apparently the orientation of the benzene ring in phenylalanine becomes more polarizable during an in-plane ring deformation. However, the 620-cm^{-1} out-of-plane ring vibration also appears with more intensity in the equatorial position, which suggests some anomaly here. The explanation might be that the 620-cm^{-1} band is the only phenylalanine band that is depolarized; the others are strongly polarized. Thus some other coupling mechanism must be present, causing depolarization and intensity (orientation) changes or simply symmetry (local) effects at the molecular site in the lens. Only phenylalanine bands appear to change appreciably with different orientation. In general, however, the changes that occur for the three orientations are small and do not represent significantly different optical characteristics for axial or equatorial position.

Lens Temperature

The high-temperature lens spectra are shown in Figures 4 and 5. Figure 5 is a recording made without the polarizer and polarizer scrambler; these were removed since all polarization had been lost. For a discussion of temperature effects on the lens, the spectral range of 200-4000 cm^{-1} can be divided into a low and high frequency region. In the 2000 to 4000-cm^{-1} range, a dramatic change is seen in the disappearance of the water; only a weak band at 3280 cm^{-1} remains. This appears reasonable since most of the water has evaporated, leaving only a residual of tightly bound hydrogen-bonded water. A new band does appear at 3115 cm^{-1}, which is most likely a C-H stretch uncovered by the decrease in water-band intensity. Another major change is seen in the complete loss of polarization effects. This indicates that a random array of protein molecules is now fixed in a solid matrix.

In the 200- to 1800-cm^{-1} region, the most obvious change is in the complete loss of polarization of all bands and the general increase of band intensity, mainly due to the drastic decrease in water concentration. The low frequency bands between 400 and 700 cm^{-1} have changed very little except for some sharpening. The tryptophan bands at 759 and 870 cm^{-1} have decreased in intensity relative to the bands at 830 and 855 cm^{-1}. The bands at 935 and 955 cm^{-1} have both become more distinct, and the 935-cm^{-1} band has slightly decreased. Another decrease is seen in the 1070-cm^{-1} (C-N stretch) band which has also shifted to 1080 cm^{-1}. The Amide III band at 1235 cm^{-1} has broadened and become more intense, especially relative to the 1207-cm^{-1} band of phenylalanine. The broadening possibly suggests more extensive hydrogen bonding. A rather large increase in band intensity is seen for the CH_2 deformation at 1445 cm^{-1}; in fact, for the band envelope between 1150 and 1500 cm^{-1}, the Raman scattered intensity has increased overall. Perhaps the most interesting and important observation is that the Amide I vibration at 1669 cm^{-1} has not changed in frequency position, although a large increase in intensity is seen in the heated spectrum. This suggests no observable change in protein conformation due to the thermal insult.

CONCLUSION

We have shown that regardless of a rabbit lens orientation with respect to incident/scattered exciting laser beams, the resultant polarized Raman spectra

are essentially identical. A few of the bands changed slightly from an axial to equatorial orientation (a local effect), but their polarization characteristics remained fairly constant. Thus, the Raman spectral results cannot be treated as originating from a true uniaxial crystal. The results indicate that the lens is optically isotropic. This is evidenced by the small depolarization ratios for all intense bands in the spectra, indicating totally symmetric vibrations. Temperature effects at 100°C seem to have no effect on protein conformation within the lens, which implies that lenticular proteins are very heat stable. These effects are observed with rabbits and may or may not be applicable to humans.

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TABLE 1. OBSERVED FREQUENCIES, RELATIVE INTENSITIES, AND ASSIGNMENTS FOR THE NORMAL RABBIT LENS: AXIAL POSITION

Frequency (cm^{-1})/ Polarization		Relative Intensity (arbitrary units)	Assignment	
		2000-4000 cm^{-1} -- low-gain intensities (Fig. 1)		
3450[a]		8.3	water	
3400	p	41.0	water	
3280	p	43.0	water	
3060	p	31.1	C-H	
3040[a]		4.7	C-H	
2970[a]		13.0	C-H	stretch
2935	p	89.0	C-H	
2870	p	40.2	C-H	
2765	p	3.0	-	
2720	p	3.0	-	
2575	p	2.5	S-H	stretch
		200-1800 cm^{-1} -- high-gain intensities (Fig. 1)		
1669	p	65.2	Amide I	
1615	dp	28.4	Tyr	
1603	dp	25.0	Phe	
1583	p	15.4	Phe	
1577	p	15.8	Trp	
1548	p	27.7	Trp	
1445	dp	36.5	CH_2 def. (Ref. 6)	
1400	p	25.1	-	
1360	p	28.1	Trp	
1340	p	38.5	Trp	
1235	p	51.0	Amide III	
1207	p	46.9	Phe	
1174	p	21.7	Tyr	
1157	p	16.5	C-N	
1125	p	22.0	C-N	stretch
[illegible]0	p	25.7	C-N	
1030	p	39.5	Phe	
1003	p	81.2	Phe	
955	p	23.6	C-C	
935	p	24.0	C-C	stretch
870	p	27.0	Trp	
855	p	31.0	Tyr	
830	p	26.5	Tyr	
759	p	24.0	Trp	
644	dp	7.0	Tyr	
620	dp	4.2	Phe	
570	p	2.0	-	
547	p	2.1	-	
492	p	2.3	Cys-Cys (Ref. 6)	
420	p	1.2	-	

[a]Bands that appeared only in the perpendicular polarization.
p = polarized; dp = depolarized.

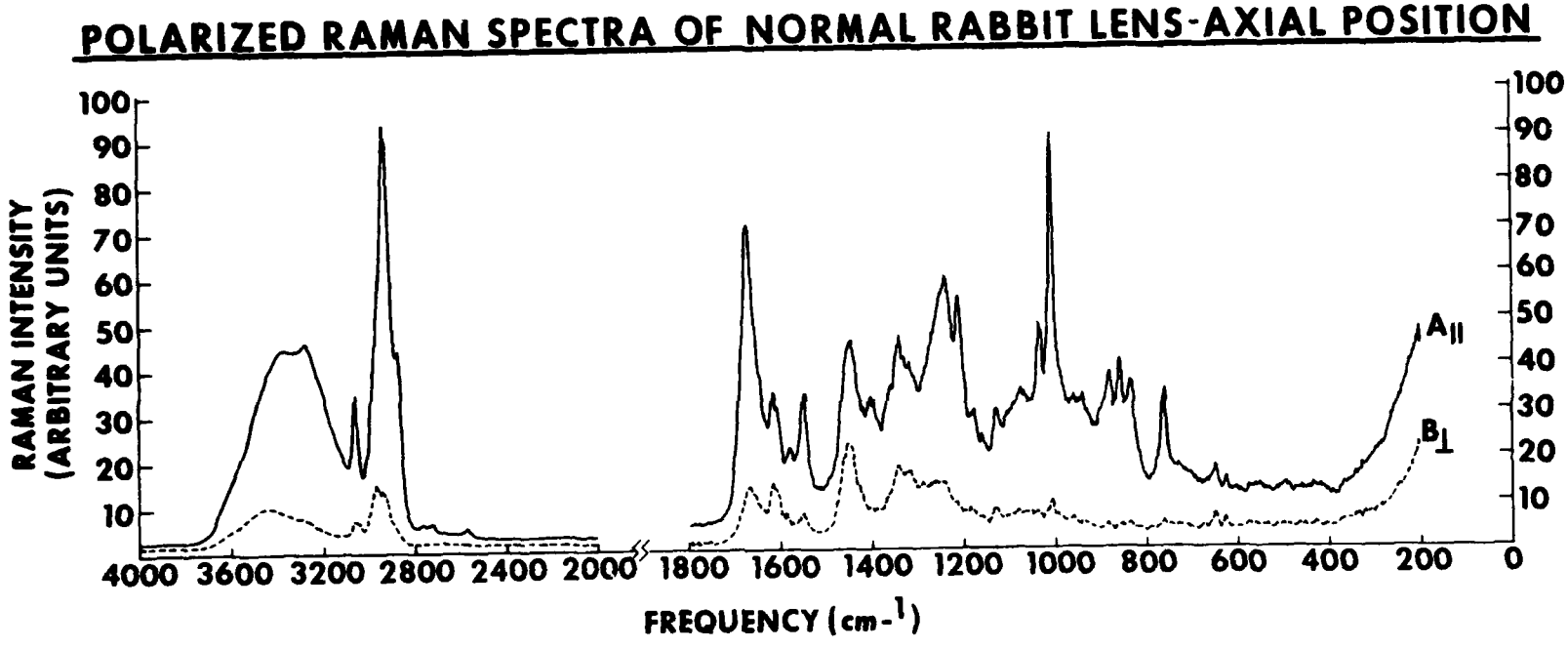

Figure 1. Polarized Raman spectra of normal rabbit lens; axial position. Instrument settings: laser power at sample = 235 mW; spectral bandwidth = 6 cm^{-1}; gain = 20,000 counts/s (200-1800 cm^{-1}), 90,000 counts/s (2000-4000 cm^{-1}); pen period = 0.5 s; scan speed = 5 cm^{-1}/s.

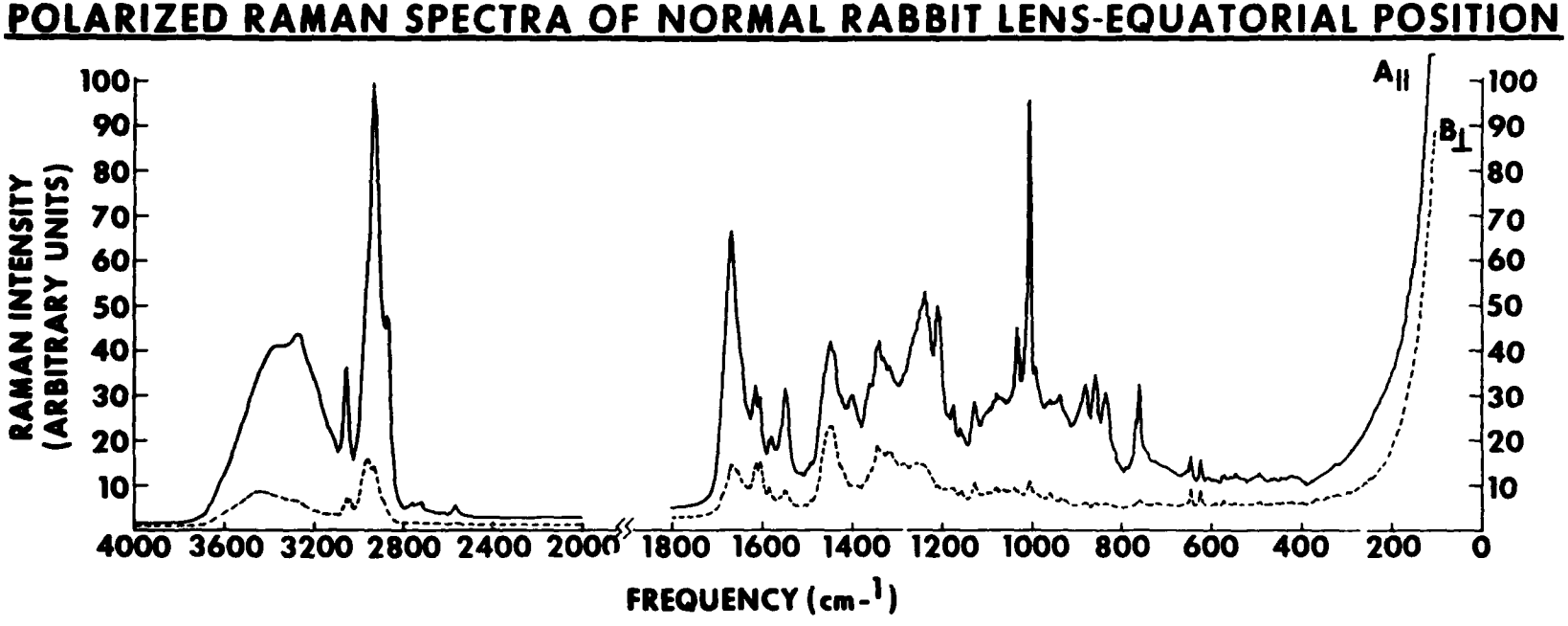

Figure 2. Polarized Raman spectra of normal rabbit lens; equatorial position. Instrument settings: laser power at sample = 235 mW; spectral bandwidth = 3 cm^{-1}; gain = 10,000 counts/s (200-1800 cm^{-1}), 50,000 counts/s (2000-4000 cm^{-1}), pen period = 1 s; scan speed = 1 cm^{-1}/s.

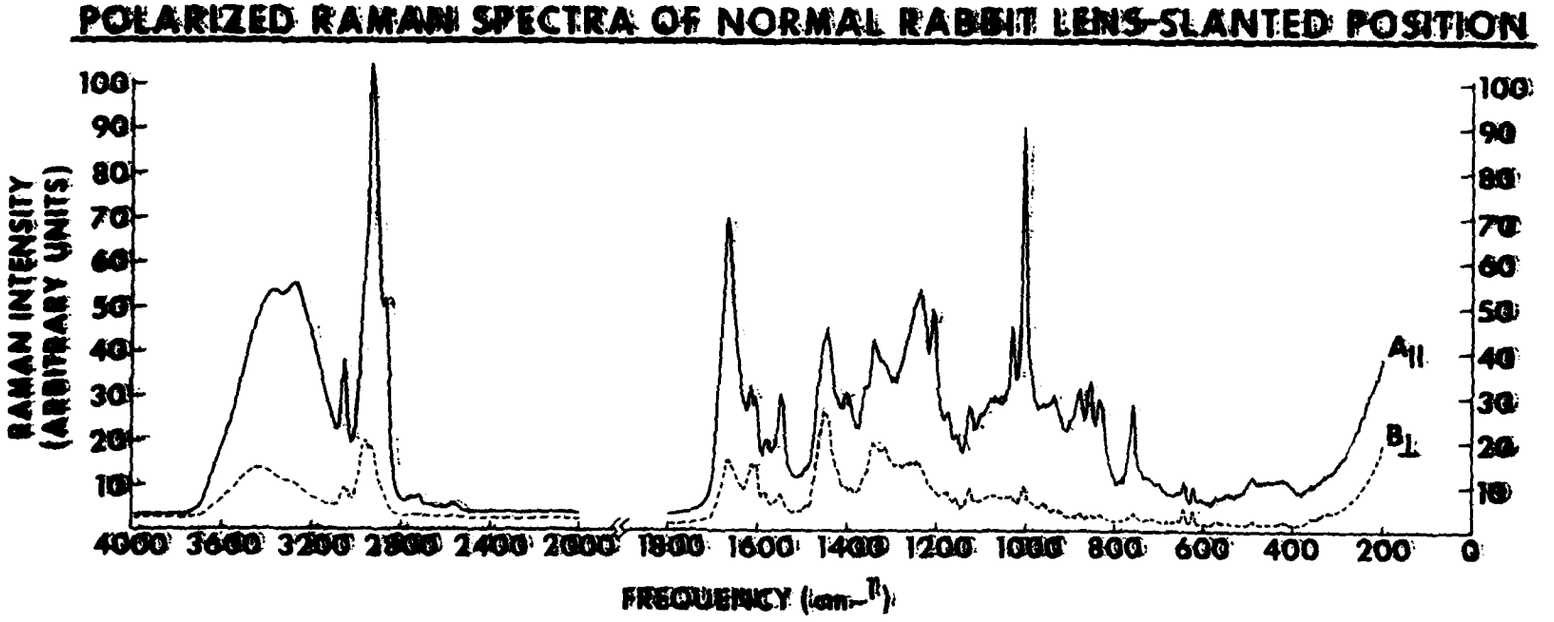

Figure 3. Polarized Raman spectra of normal rabbit lens; slanted position. Instrument settings: laser power at sample = 235 mW; spectral bandwidth = 6 cm^{-1}; gain = 32,500 counts/s (200-1800 cm^{-1}); 80,000 counts/s (2000-4000 cm^{-1}); pen period = 1 s; scan speed = 1 cm^{-1}/s.

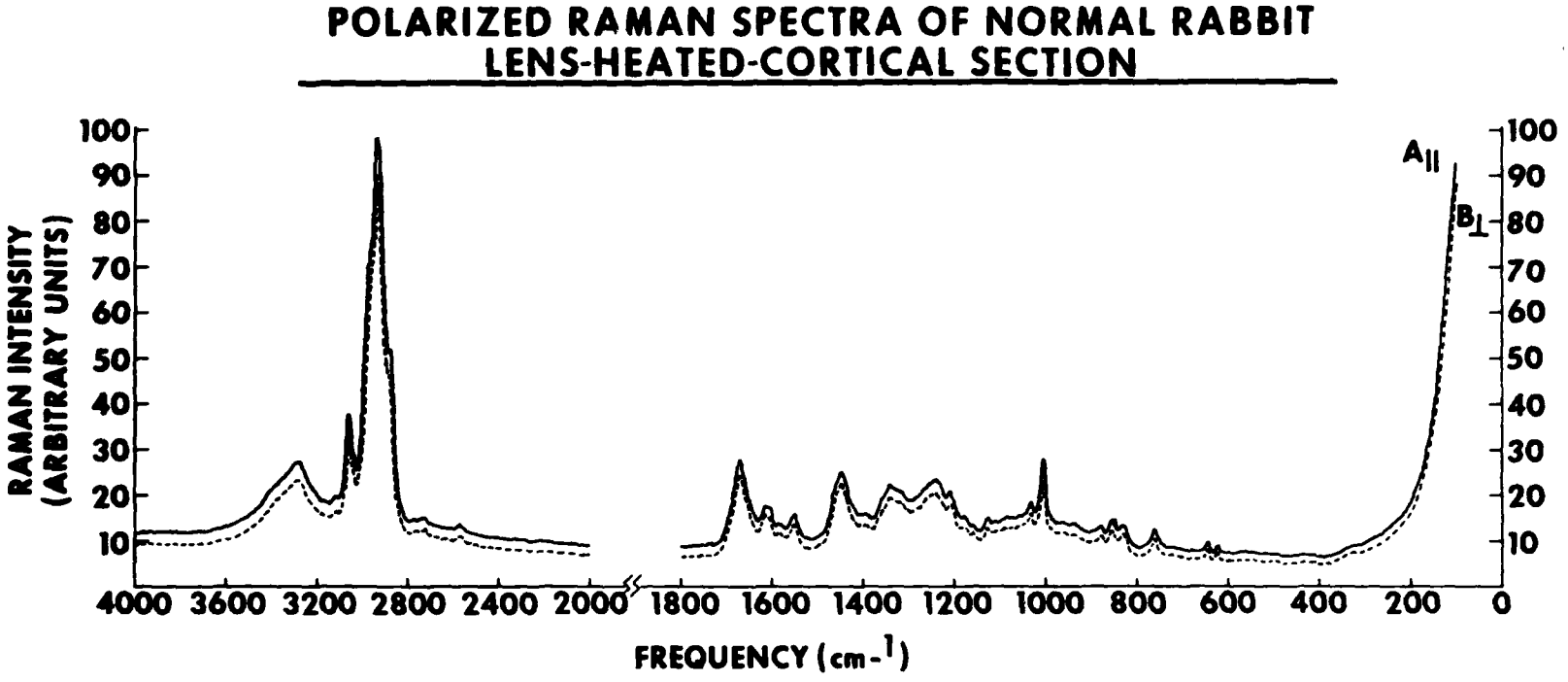

Figure 4. Polarized Raman spectra of normal rabbit lens; heated. Instrument settings: laser power at sample = 300 mW; spectral bandwidth = 6 cm^{-1}; gain = 150,000 counts/s; pen period = 1 s; scan speed = 5 cm^{-1}/s.

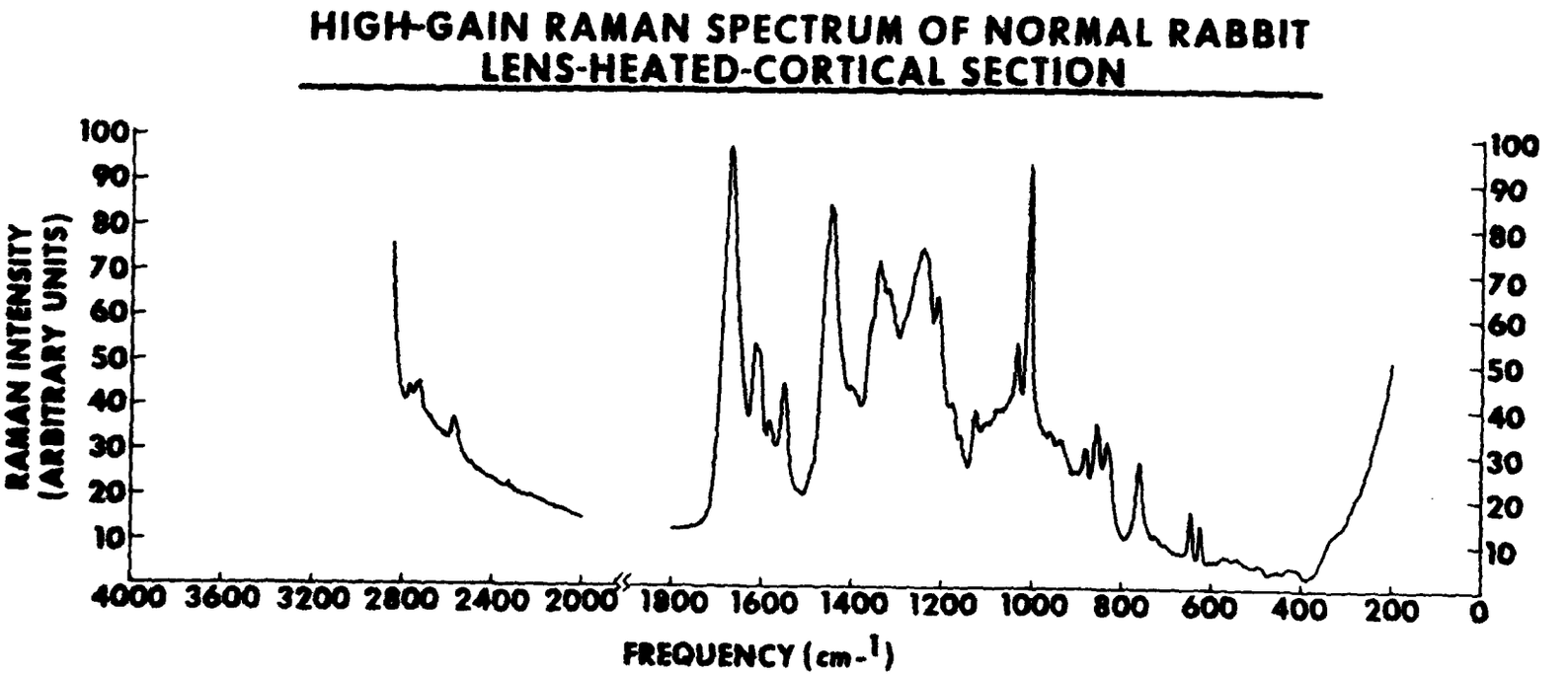

Figure 5. High-gain Raman spectrum of normal rabbit lens; heated. Instrument settings: laser power at sample = 300 mW; spectral bandwidth = 6 cm^{-1}; gain = 100,000 counts/s; pen period = 2 s; scan speed = 1 cm^{-1}/s.


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